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Flowering Time: A Pathway that Begins at the 3′ End

Dispatch

Richard M. Amasino

Flowering time control in plants involves integration of multiple signals. One of the signalling pathways in *Arabidopsis* involves a negative autoregulatory loop, in which the FCA protein together with FY promotes the choice of an alternative polyadenylation site within the FCA pre-mRNA to produce a transcript that does not encode a functional protein.

The control of flowering time has been intensively studied by genetic analyses in several plant species, particularly *Arabidopsis thaliana* [1,2]. These studies have revealed many of the genes involved in regulating flowering, but the biochemical processes in which the products of these flowering-time genes participate are largely unknown. Simpson *et al.* [3] have recently reported that two flowering-time regulatory proteins, FCA and FY, interact to control the choice of site of 3′ mRNA cleavage and polyadenylation. Furthermore, a target of the FCA–FY complex is the pre-mRNA for one of the components of the complex: FCA, a promoter of flowering (the delayed flowering of an *fca* mutant is illustrated in Figure 1).

Precisely when to initiate flowering is a critical developmental 'decision' in a plant's life cycle. Annual plants typically initiate flowering only once and then die after setting seed. Thus, the timing of this decision in annuals such as *Arabidopsis* is extremely important. The pathways that control flowering in *Arabidopsis* have evolved to provide considerable flexibility. For example, there is a photoperiod pathway that promotes flowering when the days are long. The long days of late spring and early summer are usually an optimal time for *Arabidopsis* to complete its life cycle. In short days, flowering is delayed, but the *Arabidopsis* plant continues to grow as it awaits more optimal conditions.

Two other pathways, the vernalization and autonomous pathways, regulate flowering primarily by controlling the level of expression of *FLOWERING LOCUS C* (*FLC*) [1,2]. FLC is a repressor of flowering and a member of the MADS-domain family of proteins, which are known to act as transcriptional regulators [4–6]. The autonomous and vernalization pathways both promote flowering by repressing *FLC* expression, but they do so under different circumstances.

Vernalization is the promotion of flowering that results from the prolonged exposure to cold during winter [7]. Certain varieties of *Arabidopsis* known as winter-annuals typically begin growing in the fall, but are prevented from flowering before the onset of winter by high levels of *FLC* expression. During winter the vernalization pathway represses *FLC* expression and thus permits flowering in the spring [7].

Another pathway that regulates *FLC* expression is known as the autonomous pathway because it appears to regulate flowering independently of environmental cues such as day-length and cold [1,2]. Six genes for components of this pathway have been identified, all of which affect flowering by repressing *FLC* [4]. Analyses of double mutants among pathway members, however, indicates that these genes are likely to operate in separate parallel pathways to regulate *FLC* [1,2,8]. One of these parallel pathways is defined by *FCA* and *FY*.

FCA was cloned by Caroline Dean's group, and they found that the *FCA* pre-mRNA is processed into four distinct transcripts [9]. They subsequently showed that only one of the transcripts (referred to as γ) is able to promote flowering; another major transcript (β) is generated by cleavage and polyadenylation at a site within the third intron [10,11]. This processing of the *FCA* pre-mRNA into active and inactive forms is regulated during development; this regulation is conserved in other plant species, and so is likely to be an important component of the developmental regulation of flowering time [10,11].

Recent studies have identified two of the factors involved in selection of the *FCA* pre-mRNA polyadenylation site. Quesada *et al.* [11] found that one of the factors is FCA — the FCA protein negatively regulates its own expression by favoring polyadenylation at the third intron site, which results in a transcript that does not make a functional protein.

How does FCA accomplish this negative autoregulation? An important clue was provided in the sequence of the FCA protein. Simpson *et al*. [3] noted that FCA has an RNA-binding domain and a specific type of WW domain that was predicted to interact with a Pro-Pro-Leu-Pro sequence. Such a (coding) sequence was

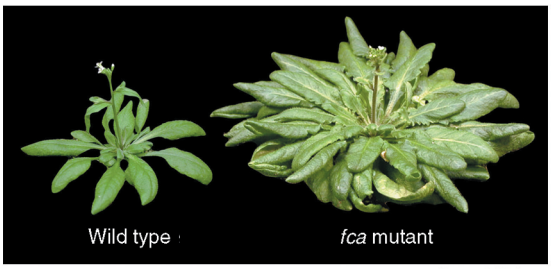

Figure 1. Phenotype of a wild-type *Arabidopsis* plant and an *fca* mutant in the Columbia accession.

The plants were grown in long days which are inductive for flowering. Both plants are shown at the time of initiating flowers and thus the *fca* mutant in which flowering is delayed is chronologically much older than the wild type. (Photograph by Scott Michaels.)

Department of Biochemistry, University of Wisconsin, 433 Babcock Drive, Madison, Wisconsin 53706-1544, USA. E-mail: amasino@biochem.wisc.edu

found in a gene in the chromosomal interval in which *FY* resides, and subsequent experiments showed that this gene was in fact *FY*. The FY protein is similar to the yeast polyadenylation factor Psf2p. Simpson *et al.* [3] went on to show that FCA and FY interact via FCA's WW domain.

The interaction of an RNA-binding protein with a polyadenylation factor suggests a possible biochemical mechanism for *FCA* autoregulation (Figure 2). The FCA protein might bind to the *FCA* pre-mRNA and, via its association with FY, direct the cleavage and polyadenylation machinery to the processing site in the third intron to favor the formation of the β transcript.

One intriguing aspect of this work is that both the budding yeast *Saccharomyces cerevisiae* and the plant *Arabidopsis* have only a single copy of the FY/Pfs2p factor. In yeast, mutation of Pfs2p is lethal [12]. Why are *fy* mutations not lethal in *Arabidopsis*? One possibility, as noted in Simpson *et al.* [3], is that the two *fy* alleles tested so far are not nulls; complete loss of *FY* function may be lethal. Another possibility is that *FY* is reserved for processing of specific regulated mRNAs. Unlike yeast, in which Pfs2p is the only protein of this type in the core cleavage and polyadenylation machinery, *Arabidopsis* also has three genes encoding proteins similar to mammalian CstF50 which might have the same role as FY/Pfs2p. CstF50 is related to FY/Pfs2p in domain organization and has been proposed to be the functional equivalent of Pfs2p in mammals [12]. Moreover, the mammalian ortholog of Fy/Pfs2p, WDC146, has not been found in the core cleavage and polyadenylation machinery. Thus, as noted [3], in *Arabidopsis* and mammals perhaps CstF50-type proteins participate in most 3′ mRNA end formation, whereas FY and WDC146 participate in regulated 3′ mRNA end formation.

These results suggest many future experiments. Does the FCA protein actually bind to *FCA* pre-mRNA as predicted? Is FY involved in the processing of certain pre-mRNAs independently of FCA? The fact that *fy fpa* double mutants are lethal, whereas *fca fpa* double mutants are not [8], indicates that FY has roles in addition to flowering that are not shared with FCA. How do FCA and FY down-regulate *FLC* expression? There is no direct evidence that *FLC* is a direct target of a FCA–FY complex or that the FLC pre-mRNA can be processed at alternative polyadenylation sites, but, as noted [3], alternative polyadenylation sites may be difficult to detect if non-functional *FLC* transcripts are rapidly degraded.

Regardless of whether or not *FLC* pre-mRNA is a direct target of the FCA–FY complex, FCA and FY acting together must somehow regulate other flowering time genes in addition to *FCA*, and a likely candidate is *FLC* (Figure 2). This is because the *fy* mutation suppresses the early flowering effect of increased production of the active FCA protein translated from the γ mRNA. If the only function of the FCA–FY complex was to negatively regulate *FCA*, which is a promoter of flowering, *fy* mutants would be earlier flowering rather than delayed in flowering.

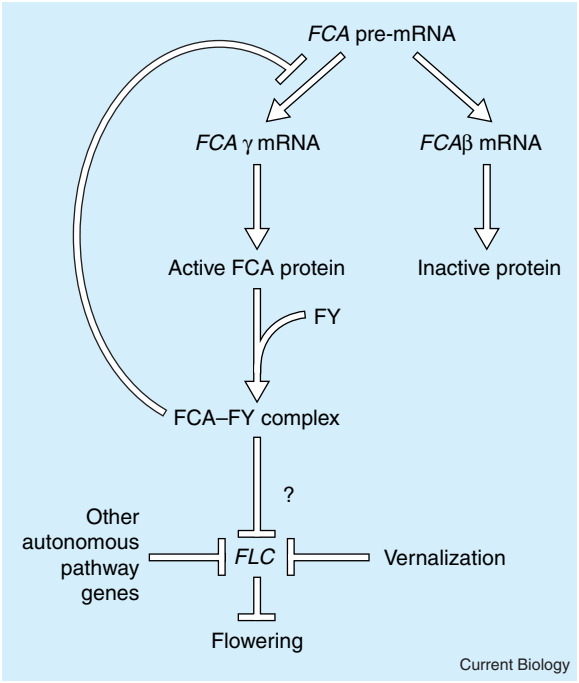

Figure 2. Model for *FCA* auto-regulation and the regulation of flowering time.

Negative autoregulation of *FCA* results when FCA and FY proteins together promote the formation of the β form of the *FCA* mRNA. The β form results from cleavage and polyadenylation at a site in the third intron and cannot produce an active protein. FCA and FY also co-operate to lower the levels of *FLC* mRNA and promote flowering. The '?' between FCA–FY and *FLC* indicates that the mechanism of this interaction is not known and may not be direct.

Raising many questions for future research is, of course, a sign of an exciting advance. The new work of Simpson *et al.* [3] is significant not only because it is a major advance in our understanding of flower-time regulation, but it is also the first example of a specific factor that regulates the choice of the cleavage and polyadenylation site of a specific gene.

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